

Soil carbon mineralization as affected by water content and nitrogen rate after ryegrass incorporated into soil

Meijuan Lu¹, Jiancheng Xu², Xiaowei Wang², Wenting Yang^{2*}

¹ College of Territorial Resources and Environment, Jiangxi Agricultural University, 330045, Nanchang, China

² Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Jiangxi Agricultural University, 330045, Nanchang, China

*Correspondence: wt yang@jxau.edu.cn

Abstract

lanting cover crop has been suggested as a way of increasing soil organic carbon in agricultural land. Ryegrass (*Lolium multiflorum* L.), as a cover crop, could improve soil fertility and lower soil CO₂ emission. However, effects of soil water content and nitrogen on soil carbon mineralization after ryegrass incorporation are not fully understood. The present study was to investigate the effect of soil water content and nitrogen rate on soil carbon mineralization after ryegrass incorporated into upland red soil (Ferralsols). A laboratory experiment was established, including soil water contents [15% (W1), 30% (W2), 45% (W3)] and nitrogen rates [0 (N1), 60 mg/kg(N2), 120 mg/kg(N3)]. The results showed that the highest soil carbon mineralization accumulation was observed in W3N3. Nitrogen application inhibited carbon mineralization rate and accumulation in the late stage of ryegrass incorporation at W1, but increased carbon mineralization rate and accumulation at W2. With increasing soil water content, nitrogen application could improve soil carbon mineralization at the early stage of ryegrass incorporation. In conclusion, soil nitrogen and water content could regulate soil carbon mineralization. Considering to reduce the soil CO₂ emissions, rational nitrogen application should be taken seriously during cover crop (ryegrass) incorporated into the upland red soil.

Key words: Ferralsols, carbon mineralization rate, accumulation, first-order kinetic equation

1 INTRODUCTION

oil organic carbon has a significant important role in the global carbon cycle(Bailey et al. 2018). Soil carbon decomposition and sequestration is mainly affected by cropping systems(Moreno-Cornejo et al. 2015), tillage methods, cover crops(Poeplau and Don 2015), organic manure and chemical fertilizer(Lehmann and Kleber 2015) in field. Soil carbon mineralization is limited by environmental factors, such as field moisture capacity(O'Connell et al. 2016), temperature(Giardina and Ryan 2000), climate(Luo et al. 2017), etc. Soil water content impacts soil oxygen, which would inhibit soil microbial activity, and finally affected soil carbon mineralization(Rivas et al. 2014). The influences of exogenous nitrogen input on soil carbon mineralization varied with environmental factors(Coucheney et al. 2013). The major objects of present documents are forest (Fisk et al. 2015), wetland(Zheng et al. 2018), grassland(Poeplau et al. 2017) and cropland(Das et al. 2019), but there are few reports about effects of soil water content and nitrogen rate on soil carbon mineralization after cover crop incorporated into red soil.

ed soil is the third most important soils of the world covering 13% of the land area(Baligar et al. 2004). Meanwhile, red soil is an important land resource in south China because of the great potential for agricultural production. However, there are some problems remained, such as uneven distribution of precipitation in space-time, serious soil and water loss, land degradation, excessive fertilizer application and so on(Zhang et al. 2013). There are highly leached and serious acidification, and have a low organic matter and nutritional deficiencies(Wilson et al. 2004). Whereas, the red soil region belongs to subtropical areas in China, which has abundance of natural resources, such as light, temperature and water, for planting cover crops in winter.

over crops had the ability to scavenge nutrients, and thus can decrease nitrate leaching(Carey et al. 2018), alleviate soil erosion(Gómez et al. 2018) and improve soil organic matter(Wolff et al. 2018). Ryegrass, as a forage grass or cover crop, has high cold resistance, high biomass production, and rich nutritional value. Sowing ryegrass could mitigate nitrous oxide emissions(Pilecco et al. 2019) and remediate contaminated soil(Li et al. 2020). Ryegrass incorporation could improve crop yield and soil quality in paddy field(Yang 1996), ryegrass residues could also lower soil CO₂ emissions and increase soil C stocks(Mwafurirwa et al. 2019). Soil carbon mineralization is an important indicator for measuring soil quality(Gil-Sotres et al. 2005). Planting ryegrass as cover crop after sweet corn harvested in late October, and incorporated into soil in next early April, was it beneficial to improve soil quality? Our results showed that applied chemical nitrogen fertilizer (60 mg/kg) could inhibit ryegrass decomposition and nitrogen release(Yang et al. 2018), and soil nitrogen mineralization(Wang et al. 2018) at the early stage of ryegrass incorporation, which might alleviate the loss of soil nitrogen. However, effect of soil water content and nitrogen rate on soil carbon mineralization after ryegrass incorporation is not clear yet.

he objectives of this laboratory experiment were to study the influence of soil water content and nitrogen rate on soil carbon mineralization, and optimize soil water and nitrogen management under ryegrass incorporated into upland red soil. We hypothesized that soil water content and nitrogen rate could affect the ryegrass decomposition, and then regulate soil carbon mineralization in upland red soil.

2. MATERIALS AND METHODS

2.1 Ryegrass and soil

he ryegrass (*Lolium perenne* L.cv. ‘Ganxuan No.1’) was planted after sweet corn harvested on the 15th October, 2013, and harvested before sowing sweet corn on 1st April, 2014. The ryegrass was cut into small pieces and dried in an oven at 105 °C for 30 min, and then at 80 °C until a constant weight was reached. The main nutritional components of dry ryegrass were tested: total C 360.19 g/kg, total N 27.62 g/kg, C/N 13.04, total P 21.08 g/kg and total K 67.17 g/kg.

he experimental soil, a Ferralsols, was collected from 0-20 cm layer from Jiangxi Agricultural University Sci-tech Park in Nanchang, China (28° 45’ N, 115°50’ E). The soil was air-dried, removed visible plant roots, passed through a 2-mm sieve for the incubation study. The basic chemical properties of the soil were shown in Table 1.

Table 1. Soil chemical characteristics

Soil type	pH	Soil Organic C (g/kg)	Total N (g/kg)	Available N (mg/kg)	NH ₄ ⁺ -N (mg/kg)	NO ₃ ⁻ -N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Original soil	4.85	8.59	1.47	100.23	33.15	30.44	65.98	152.75
Incubated soil	4.65	11.01	2.05	136.17	42.53	31.49	106.19	208.41

2.2 Soil incubation

he experiment was a completely randomized design with three replicates which conducted from 11th May, 2014 to 8th August, 2014. There were 3 soil water contents [15% (W1), 30% (W2), 45% (W3)] and 3 nitrogen rates [0 (N1), 60 mg/kg (N2), 120 mg/kg (N3)]. 2.5 g dry ryegrass mixed into each 100 g soil, which was used as incubation soil. Each jar (250 ml) was packaged with 50 g incubation soil. The jars were added 7.5, 15 and 22.5 ml deionized water at W1, W2 and W3, respectively; and added 0, 14.15 and 28.30 mg ammonium sulfate ((NH₄)₂·SO₄) at N1, N2 and N3, respectively.

2.3 The process of soil organic carbon mineralization

he experiment was measured potentially mineralization Carbon using soil CO₂ flux as described by(Rabbi et al. 2014). A centrifuge tube (10 ml) after adding 5 ml of 1 N NaOH was

placed in each jar, and the jars were sealed (air-tight) with wax, then shaded cultivation at 25 °C. The tubes were collected in 1, 3, 7, 13, 21, 31, 43, 57, 73 and 91 days. The amount of CO₂-C produced during incubation was trapped in 1 M NaOH. At first, added 1 ml saturated BaCl₂ and phenolphthalein indicator into the tube. And then the amount of CO₂-C was determined by titration against 0.1 M HCl with acid burette.

2.4 Parameter simulation and data analyses

his experiment used first-order kinetics model to assess soil carbon mineralization process (Cooper et al. 2011):

$$C_t = C_0 \times (1 - e^{-kt})$$

The first-order kinetic model was simulated by Matlab7.0. Where C_t -total organic carbon at time t ; C_0 -the amount of the potential soil carbon mineralization; K -the decay constants of soil carbon mineralization rate, and t - the incubation time.

Soil carbon mineralization rate ($\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$) = Total soil carbon mineralization / days of incubation

Actual carbon mineralization rate (%) = Total soil carbon mineralization / Soil carbon content in incubated soil $\times 100\%$

Potential carbon mineralization rate (%) = Total potential soil carbon mineralization / Soil carbon content in incubated soil $\times 100\%$

Net carbon mineralization ($\text{mg} \cdot \text{g}^{-1}$) = Total soil carbon mineralization of nitrogen treatment - Total soil carbon mineralization without nitrogen treatment

Net carbon mineralization rate (%) = Net carbon mineralization / Soil carbon content in incubated soil $\times 100\%$

The statistical significance of differences among treatments were tested with ANOVA (SPSS 19.0, SPSS Inc., USA), Duncan test was used to compare the means, differences at $p < 0.05$ level were considered to be statistically significant.

3 RESULTS

3.1 Dynamic of soil carbon mineralization accumulation

oil carbon mineralization was influenced by nitrogen rate and soil water content. At W1, compared to N1, the soil carbon mineralization contents under N2 and N3 were significantly reduced from the 7th to 91st day, the soil carbon mineralization contents under N2 and N3 were decreased by 13.57% and 12.24% in the 91st day, respectively. However, compared to N1, N3 significantly increased the soil carbon mineralization contents at W2 and W3; the soil carbon mineralization contents were increased by 6.85% and 9.89% at W2 and W3 in the 91st day, respectively. There was no significant difference between N2 and N3, the highest soil carbon mineralization contents was W3N3, reached to 4.14 mg/kg (Figure 1).

Different soil water contents had different influence on the soil carbon mineralization under the same nitrogen rate. Under N1, compared with W1, the soil carbon mineralization content in W2 was drastically reduced at the 1st and 3rd day, and significantly reduced at the 31st and 43rd day in W3, but significantly increased at the 13th day. Under N2 and N3, compared with W1, adding soil water content (W2 and W3) significantly increased soil carbon mineralization appeared in the middle and later periods (7th -91st day), there was no significant difference between W2 and W3.

Figure 1. Dynamic of soil carbon mineralization

Note: The W1, W2 and W3 represent the soil water contents 15%, 30% and 45%, respectively. The N1, N2 and N3 represent the nitrogen rates 0, 60 and 120 mg/kg, respectively. Error bars indicate the standard error of the mean ($n=3$), activities with different lowercase letters at the same column indicate significant differences at $P < 0.05$.

wo-factor variance analysis indicated that nitrogen application and soil water content both significantly affected soil carbon mineralization (Table 2). With the same water content (W1), compared to N1, increasing nitrogen fertilization (N2 and N3) significantly inhibited soil

carbon mineralization amount and rate. However, there is no obviously difference between N2 and N3. Under W2 and W3, compared to N1, increasing nitrogen fertilization (N3) significantly increased soil carbon mineralization amount and rate. Meanwhile, the soil carbon mineralization amount at N3 significantly increased than that at N2. With the same nitrogen rate (N1), compared to W1, increasing soil water content (W2 and W3) didn't significantly affect soil carbon mineralization amount and rate. At N2 and N3, compared to W1, soil water content (W2 and W3) significantly increased soil carbon mineralization amount and rate. Meanwhile, soil carbon mineralization amount and rate under W3 obviously improved than that under W2.

et carbon mineralization amount and rate at N2 and N3 both had negative value under W1. Under W2 and W3, net carbon mineralization amount and rate were significantly higher at N3 than that at N2. With the same nitrogen rate (N1), net carbon mineralization amount and rate hadn't significant difference among W1, W2 and W3.

Table 2. Effect of soil water content and nitrogen rate on accumulation and rate of soil carbon mineralization

Factors	Treatments	Accumulation mineralization amount (mg/g)	Mineralization rate (%)	Net mineralization amount (mg/g)	Net mineralization rate (%)
Nitrogen rate and Soil water content	W1N1	3.75±0.02cd	21.50±0.12b	-	-
	W1N2	3.24±0.04e	18.58±0.21d	-0.51±0.06d	-2.92±0.32d
	W1N3	3.29±0.06e	18.86±0.33cd	-0.46±0.05d	-2.63±0.31d
	W2N1	3.72±0.05d	18.62±0.33d	-	-
	W2N2	3.80±0.02cd	19.17±0.26cd	0.10±0.05bc	0.55±0.31bc
	W2N3	3.98±0.03b	19.58±0.22c	0.17±0.06a	0.96±0.35a
	W3N1	3.76±0.08cd	21.60±0.46b	-	-
	W3N2	3.89±0.07bc	22.30±0.43b	0.12±0.03bc	0.70±0.14bc
	W3N3	4.14±0.02a	23.74±0.10a	0.37±0.07a	2.14±0.39a
Two-factor variance analysis (<i>F</i> value)	Nitrogen rate	4.769*	-	6.417**	-
	Soil water content	115.147**	-	103.013**	-
	Nitrogen rate × Soil water content	20.271**	-	27.270**	-

ote: Activities with different lowercase letters at the same column indicate significant differences at $p < 0.05$. * indicate significant differences at $p < 0.05$, ** indicate extremely significant differences at $p < 0.01$.

3.2 Dynamic of soil carbon mineralization rate

All the treatments had the maximum rate of soil carbon mineralization after 1st day of incubation and then dropped rapidly until the 91st day (Figure 2). soil carbon mineralization rates of N2 and N3 were significantly higher than that of N1 at W2. While at W3, compared with N1, soil carbon mineralization rate of N2 was decreased by 7.26%, but that of N3 was increased by 5.73%. In the N1, compared to W1, soil carbon mineralization rate of W2 was significantly decreased; but soil carbon mineralization rate of W3 was obvious higher than that of W2. In the N2 and N3, there was no significant difference in soil carbon mineralization rate among W1, W2 and W3. Soil carbon mineralization rate of W2 was significantly higher than that of W3 at N2. From the analysis results of Table 3, nitrogen rate and soil water content had significantly influenced on soil carbon mineralization rate.

Figure 2. Dynamic of soil carbon mineralization rate

Note: The W1, W2 and W3 represent the soil water contents 15%, 30% and 45%, respectively. The N1,

N2 and N3 represent the nitrogen rates 0 mg/kg, 60 mg/kg and 120 mg/kg, respectively. Error bars indicate the standard error of the mean ($n=3$), activities with different lowercase letters at the same column indicate significant differences at $P<0.05$.

Table 3. Two-way analysis of variance of soil carbon mineralization rate

Incubated days	Nitrogen rate	Soil water content	Nitrogen rate×Soil water content
1	12.93**	0.173	14.511**
3	8.956**	1.232	7.689**
7	8.049**	36.026**	7.313**
13	4.704*	111.088**	16.902**
21	0.688	71.872**	22.200**
31	2.917	57.272**	26.136**
43	7.791**	44.554**	33.159**
57	5.257*	99.258**	28.477**
73	5.099*	63.008**	16.726**
91	8.431**	92.652**	24.139**

ote: * indicate significant differences at $p<0.05$, ** indicate extremely significant differences at $p<0.01$.

3.3 Kinetics parameters of soil carbon mineralization

he pattern of carbon mineralization curve fitted well in first order kinetics model ($R^2=0.97-0.99$) (Table 4). From all the treatments, the highest potential mineralization amount was observed in W2N3, and the lowest one was recorded in W1N2. With the same nitrogen rate (N1, N2, N3), compared to W1, W2 significantly increased the potential mineralization amount C_0 . However, There were no obvious difference between W2 and W3 at N2 and N3. Adding soil water content and nitrogen rate appropriately could increase the potential mineralization carbon in the red soil during ryegrass incorporation.

itrogen rate and soil water content both obviously impacted on K value. The K value of W3N3 was significantly higher than other treatments. The K value of each treatment at W2 was significantly lower than those at W1 and W3. The maximum of potential mineralization rate was obtained from W2N3, and the minimum one obtained from was W1N2.

Table 4. Kinetics parameters of soil carbon mineralization

Treatments	Fitting parameters			Potential mineralization rate (%)
	Potential mineralization amount C_0 (mg/g)	Mineralization rate constant $K(d^{-1})$	R^2	
W1N1	3.89±0.17bcd	0.0271±0.0026b	0.9915	22.33±0.96cde
W1N2	3.38±0.06e	0.0267±0.0004b	0.9903	19.39±0.33f
W1N3	3.56±0.09de	0.0241±0.0005b	0.9884	20.41±0.53ef
W2N1	4.34±0.19a	0.0151±0.0009c	0.9928	24.8±1.14ab
W2N2	4.23±0.09ab	0.0168±0.0006c	0.9927	24.28±0.52abc
W2N3	4.49±0.03a	0.0155±0.0005c	0.9897	25.75±0.18a
W3N1	3.97±0.08bc	0.0246±0.0002b	0.9789	22.76±0.46bcd
W3N2	3.93±0.10bc	0.0277±0.0012b	0.9843	22.58±0.55cd
W3N3	3.62±0.12cde	0.0362±0.0015a	0.9792	20.75±0.68def
Nitrogen rate	3.183	4.82*	-	3.01
Soil water content	34.648**	108.878**	-	32.775**
	3.246*	11.673**	-	3.313*

ote: Activities with different lowercase letters at the same column indicate significant differences at

$p < 0.05$. * indicate significant differences at $p < 0.05$, ** indicate extremely significant differences at $p < 0.01$.

4 DISCUSSION

The present work indicated exogenous nitrogen addition inhibited soil carbon mineralization under low soil water content (15%). Soil microorganisms would be given priority to aerobic respiration, decomposed small molecule matter, enhanced microbial activity, and accelerated the mineralization efficiency (Ross et al. 2013). Low soil water content might inhibit the soil enzyme activity (Marschner and Kalbitz 2003) and microbial activity (Linn and Doran 1984), meanwhile, overuse mineral N could increase the aromaticity and or complexity of DOM molecules (Michel et al. 2006), those might mitigate the mineralization of soil organic matter.

Increasing soil water contents (30 and 45%) with nitrogen fertilizer application could improve soil carbon mineralization in the present results. The similar results were reported in wheat-maize cropping system (Kan et al. 2020), floodplain wetlands (Yin et al. 2019) and Wuyi mountains (Xu et al. 2019). Increasing nitrogen fertilization could improve soil carbon mineralization during ryegrass incorporation with the soil water contents (30 and 45%). The reasonable soil moisture could increase microbial activity (Linn and Doran 1984), and improve rates of aerobic heterotrophic respiration (Das et al. 2019), then increase soil carbon mineralizability. Nitrogen fertilizer could increase soil carbon mineralization compared to without nitrogen fertilizer (Wang et al. 2020). The initial N concentration of the residues exerted a great influence on C mineralization. High N content supplied available N to soil microorganisms in short term and stimulate microbial activity (Ding et al. 2018). Residue with high N concentrations and low C/N ratio could accelerate the initial C mineralization (Raiesi 2006), those were benefit to soil organic carbon mineralization.

The high soil carbon mineralization rates were all observed in the early stage (the first 7 days) after ryegrass incorporation. This might be sufficient carbon and nitrogen resource in soil and ryegrass was rapidly degraded, that provided the fundamental materials for the reproducing of soil microorganisms during the early period. In the present work, nitrogen application inhibited the rate of soil carbon mineralization at low soil water content. The soil enzyme activity is a key factor in the decomposition of soil organic matter, it might limit by low soil water content (Marschner and Kalbitz 2003). As the soil water content increasing, adding nitrogen fertilizer significantly improved the soil carbon mineralization rate. This result was consistent with Li et al (2014). Previous studies found that increasing water content could improve soil carbon mineralization rate with 45% to 75% upland field capacity (Norton et al. 2012). Reasonable soil water content might promote the soil microbial and enzymatic activities (Galantini and Rosell 2006), and nitrogen fertilizer supply more available N (Ding et al. 2018), both leading to higher rate of soil carbon mineralization.

In our incubation experiment, the first-order kinetic equations were better fit SOC mineralization processes. it was similar with previous report (Yin et al. 2019). In the present work, soil water content significantly influenced the potential carbon mineralization. Potential mineralizable C (C_0) determined from the equation which is a measure of active or labile and easily decomposable SOC. These parameters might be used to determine the effect of agricultural practice and tillage on C sequestration and short term nutrient turn over or fertility (Doyle et al. 2004).

5 CONCLUSIONS

Nitrogen application significantly inhibited the soil carbon mineralization in the late stage of ryegrass incorporation at 15% soil water content, but increased the carbon mineralization at 30% soil water content. Without nitrogen application, increasing soil water content significantly inhibited the soil carbon mineralization at the early stage of ryegrass incorporation. However, soil water content increased the soil carbon mineralization with nitrogen applied. In a conclusion, nitrogen fertilizer application and soil water content significantly affected the soil carbon mineralization after ryegrass incorporated into red soil. In order to improve soil carbon sequestration and reduce greenhouse gas emission, rational nitrogen application should be taken seriously during cover crop (ryegrass) incorporated into

the upland red soil.

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Conflicts of Interest: The authors declare no conflict of interest.

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